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Dated: January 27, 2003 Signature: Staci Harris (Staci Harris)

Docket No.: HO-P02191US0 (PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Per Andersson, et al.

Application No.: 09/674,457 Group Art Unit: N/A

Filed: May 7, 1999 Examiner: N/A

For: MICROFLUIDIC DEVICE

CLAIM FOR PRIORITY AND SUBMISSION OF DOCUMENTS

Commissioner for Patents
Washington, DC 20231

Dear Sir:

Applicant hereby claims priority under 35 U.S.C. 119 based on the following prior foreign application filed in the following foreign country on the date indicated:

Country	Application No.	Date
UNITED KINGDOM	9809943.5	05/08/1998

In support of this claim, a certified copy of the said original foreign application is filed herewith.

Dated: January 27, 2003

Respectfully submitted,

By _____
Melissa W. Acosta
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I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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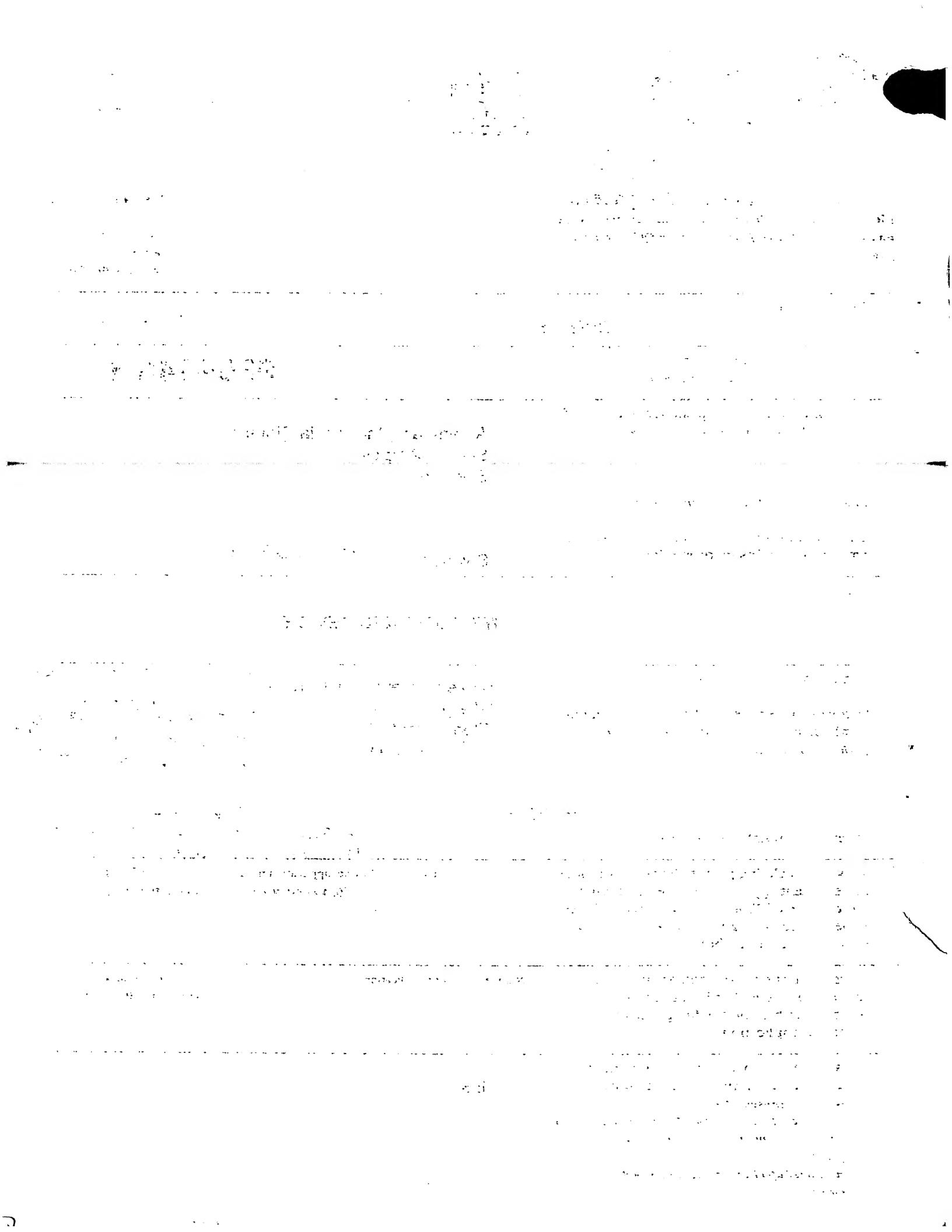
Signed Stephen Hordley

Dated 15 January 2003



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Request for grant of a patent

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Cardiff Road
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1. Your reference

JCB/2148

08 MAY 1998

2. Patent application number
(The Patent Office will fill in this part)

9809943.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Amersham Pharmacia Biotech AB,
S-751 82 Uppsala,
Sweden.

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

Sweden 7433352 001

4. Title of the invention

MICROFLUIDIC DEVICE

5. Name of your agent *(if you have one)*

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
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See note (d))

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Continuation sheets of this form	
Description	11
Claim(s)	2
Abstract	
Drawing(s)	4

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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Stevens, Hewlett & Perkins Date **08/05/98**

Stevens, Hewlett & Perkins

12. Name and daytime telephone number of person to contact in the United Kingdom

J.C. BOYDELL 0171-936 2499

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"MICROFLUIDIC DEVICE"

The present invention relates to microfluidic devices which may be used for a variety of biological processes, e.g. screening putative biologically active molecules against cell cultures or separating biological materials, the preparation of such devices and their use.

PCT patent application 97/21090 describes a microanalytical/ microsynthetic system for biological and chemical analysis which comprises a rotatable microplatform, for example a disk, having inlet ports, microchannels, detection chambers and outlet ports through which fluid may flow.

It has now been found that microfluidic devices can be prepared in which fluid flow may be controlled by having different surfaces of the substrate forming the device having different surface characteristics. By "microfluidic devices" is meant devices that can handle microvolumes of reagents, for example samples of less than 1μl, preferably between 1 and 10 nl, may be introduced into the device. By "fluid" is meant dry powders and liquids, including suspensions of particulates in liquids.

Accordingly, in a first aspect the present invention provides a microfluidic device adapted such that the flow of fluids within the device is controlled by different surfaces of the device having different surface characteristics.

The nature of the surface characteristics which control fluid flow is dependent upon the nature of the fluid itself. For example, when the fluid is a liquid, the surface characteristic that controls the flow of the liquid is preferably the surface energy of the material, e.g. low energy surfaces are normally hydrophobic whilst high energy surfaces are normally hydrophilic. The energy of a surface may be measured in terms of the critical surface tension(see for example Surface and Interfacial Aspects of Biomedical Polymers, Vol I, Plenum Press, New York, 1985, Ch.7). When the fluid is

particulate, the surface characteristic that controls the flow of the particles is dependent upon the nature of the particles, e.g. the surface is treated to interact with the particle, for example if the particle carries a charge the surface will have the same or opposite charge, similarly if the particle is magnetic the surface may be permanently or transiently magnetised.

In one embodiment there is provided a microfluidic device comprising a substrate whose surface is treated to provide areas having different surface characteristics, said areas being arranged to enable control of the flow of fluids passing across the substrate. For example, the substrate may have a hydrophobic surface interspersed with a plurality of hydrophilic areas. Alternatively, the substrate may have a hydrophilic surface interspersed with a plurality of hydrophobic areas.

Preferably, the device has a second substrate approximately parallel to the first; the first, and optionally the second substrates having surface areas of different surface characteristics that control the flow of fluid within the device.

When the substrate comprises a hydrophobic surface interspersed with hydrophilic areas, these hydrophilic areas suitably comprise a plurality of arrays of hydrophilic spots on the hydrophobic surface. By an array of spots is meant a number of spots, suitably greater than 10 and preferably greater than 50, for example 200 , which are arranged on the surface within the same fluid pathway in a predetermined pattern. The array may be single dimensional – i.e. a line of spots, or multi-dimensional.

By areas of different surface characteristics is meant that areas of the surfaces of the substrate have different relative characteristics, for example, in the case of liquids, different relative hydrophobicities or hydrophilicities. Boundaries between such areas may in effect form "walls" defining the flowpath of fluid within the device. Alternatively, they may form "valves" preventing the flow of fluid across the boundary until the fluid has either been provided with sufficient energy to enable it to

overcome the difference in surface energies of the surfaces or, if the characteristic of the surface can be imparted to the surface transiently, e.g. in the form of an electric charge, magnetic field, particular temperature or light intensity, by changing the characteristic of the surface.

It is believed that the terms hydrophobic and hydrophilic are well known to those skilled in the art. That a surface is hydrophobic means that water does not spread on it but stands up in the form of droplets the contact angle being that measured from the plane of the surface, tangent to the water surface at the three phase boundary line. Thus, hydrophobic surfaces have been characterised as having high contact angles with water, often in the range 40 to 110 degrees (Zettlemeyer, Hydrophobic Surfaces, Ed.F.M.Fowkes, Academic Press, (New York). Hydrophilic surfaces are those which have low contact angles with water, often in the range 1 to 25 degrees. However, without limitation and for the purpose of guidance only, suitable hydrophobic surfaces include hydrocarbon polymers, including halogenated hydrocarbon polymers, see for example table 1; whilst suitable hydrophilic surfaces include non-contaminated metal oxides, silicaceous materials, such as glass and polysaccharides. Surfaces of materials may be modified to change their properties, i.e. hydrophilic materials may be given hydrophobic properties by surface treatment with a hydrophobic material such as hydrocarbon, perfluorinated hydrocarbon or silicone containing species. Likewise, hydrophobic materials can be made hydrophilic by the introduction of charged groups or hydroxyl, amide or polyether groups on the surface. A small fraction of a monomolecular layer may be sufficient to change the surface characteristics drastically. When the hydrophobic/hydrophilic boundaries form "walls" and "valves", then the surface energy difference to form a wall may be the same or different to that for a valve, however the energy difference for a wall will normally be higher than that for a valve.

Some or all of the areas interspersed on the surface (be they

hydrophobic or hydrophilic) may suitably be treated to allow the culture of cells on them. In this embodiment the device may for example be used for screening intracellular events (see for example European Patent 650396 B on how this may be performed).

Suitable liquids for use in the devices of the present invention are those which have a surface tension preferably greater than $18mNm^{-1}$. Aqueous solutions or suspensions which have a surface tension greater than $50mNm^{-1}$ are preferred.

Suitable particulates for use in the devices of the present invention are powders or beads having a particle size of less than 200μm. Such powders or beads are preferably treated in some way, for example they carry an electric charge or are magnetic, that makes them more amenable to flow through the device of the present invention. Whilst the present invention anticipates the use of particulates in the devices of the present invention in the absence of a liquid carrier, they may also be present in such a liquid carrier.

The microfluidic device is preferably circular and adapted for rotation about its axis. Such adaptation may take the form of a hole at the axis of one or both substrates which is capable of engaging a drive shaft. Other methods of rotating the device include clamping the device and contacting the perimeter with a moving surface, for example moving wheels, or placing the device on a turntable and spinning the turntable.

When the device is circular the fluid inlet is normally towards the axis of the device. The inlet may be a single port attached to an annular feed channel within the device or it may be a series of ports arranged at spaced angular intervals around the axis. An annular outlet is normally located towards the circumference of the device. Fluid may flow in a laminar manner across the surface of the device or it may flow in channels formed either by hydrophobic/hydrophilic boundaries or by interior walls connecting the two substrates. These interior walls are conveniently arranged radially around

the axis of the device. The channels are normally of suitable dimensions to enable capillary forces to act upon the fluid within the channel.

When the device is adapted for cell culture it is preferable to have a source of gases available which aid cell growth. In this case, there will be one or more gas inlets in the device, which are conveniently situated in close proximity to the cells to be cultivated. Gas pathways are provided connecting the gas inlets to the cells or the fluid pathways connected to the cells, enabling culture medium/nutrients and gas, e.g. air, to be supplied down the fluid pathways.

The substrates forming the device are conveniently parallel and are preferably sufficiently close together to enable liquids in the device to be subject to capillary forces, preferably less than two millimetres apart; preferably less than one millimetre. Thus a liquid can be fed into the fluid inlet and will then be sucked down the fluid pathways by capillary action until it reaches a valve conveniently a hydrophobic/hydrophilic boundary, past which it cannot flow until further energy is applied. This energy may for example be provided by the centrifugal force created by rotating the device. Once the centrifugal force is sufficient, the liquid will flow over the valve and continue in an outward direction until it reaches the annular fluid outlet. When the areas interspersed on the surface are hydrophilic, the fluid will have a surface tension greater than $50mNm^{-1}$, for example aqueous solutions or suspensions, and when they are hydrophobic the fluid will be hydrophobic, e.g. non polar organic solvents. Thus, the fluid will be attracted to the areas/spots on the surface.

In one embodiment the areas form arrays of spots of hydrophobicities or hydrophilicities of a predetermined pattern. Such arrays can be used to build up deposits of materials to be analysed e.g. antibodies, oligonucleotides or a chemical library. For example, droplets of solvents containing the material to be analysed form on the surface, the solvent evaporates and the material is deposited.

In a second embodiment pathways are formed between parallel substrates. In this case surfaces forming the fluid pathways may themselves have areas of alternating hydrophobicity and hydrophilicity forming arrays of spots as above. These alternating areas of hydrophobicity/hydrophilicity may be formed on the surface of one or both substrates, e.g. one surface may have alternating areas whilst the opposing surface does not.

Alternatively, the fluid pathways may contain a substance for separating chemical/biological materials, e.g. a gel for chromatography or electrophoresis or beads may be trapped in the pathways for carrying out assays; for example, scintillation proximity assays or cells can be trapped in the pathways through specific surface recognition.

Areas of hydrophobicity/hydrophilicity on a surface may be formed by methods well known to those skilled in the art, for example:

1) Masking and plasma treatment

This is applicable to most surfaces and enables different degrees of hydrophilicity/hydrophobicity to be achieved with ease. A mask (adhesive tape or cast film) is attached so that it fits tightly to all the surface features. Plasma treatment is then carried out on the non-masked surface.

2) Hydrophilic "photoresist"

The plastic surface is coated with a very thin layer of hydrophilic polymer (e.g. a polylvinylcinnamate) which is crosslinked by illumination through a mask. Non-crosslinked polymer is washed off.

3) Crosslinkable surface active polymer

A surface active, reactive polymer is adsorbed from aqueous solution to the plastic surfaces and illuminated through a mask. Non-crosslinked polymer is washed off.

4) Polymerisable surfactants

A monolayer of polymerisable surfactant (e.g. the diacetylene functional phopholipids from Biocompatibles Ltd) is adsorbed and

illuminated through a mask. Non-crosslinked surfactant is washed off.

5) Photo-oxidation

The plastic surfaces are illuminated with a powerful light source (e.g. Hg lamp or uv laser) through a mask so that the illuminated areas are oxidised by atmospheric oxygen.

6) Electron beam treatment

The plastic is irradiated through a mask so that irradiated areas are in contact with air (or other reactive medium) and are oxidised creating hydrophilic groups.

In order that the invention may be better understood, several embodiments thereof will now be described by way of example only and with reference to the accompanying drawings in which:-

Figure 1 is a diagram of a surface treated in accordance with the invention;

Figures 2 and 3 are diagrams similar to Figure 1, showing different arrangements;

Figure 4 is a diagram of a twin substrate microfluidic device according to the invention;

Figure 5 is a diagram to illustrate the use of hydrophilic areas to grow cells;

Figure 6 is a partial plan view of a rotary disc microfluidic device according to the invention; and

Figure 7 is a view of part of Figure 5, illustrated in greater detail.

Referring firstly to Figure 1, there is shown a mask with an array of 6x6 hydrophilic spots 1, each of 3x3 mm on a 50x50 mm hydrophobic surface 2; which was made in Mac DrawPro and printed on a laser printer. The printout was copied on to a transparency sheet in a copying machine.

The volume of a 25 mm thick film on a 50x50 mm surface 2 is 62.5 ml. This volume polyacrylamid (PAA) was deposited on the hydrophobic side of a Gelbond film and the above mask was placed on top of the

droplet. The area under the mask was wetted by capillary forces (a small portion of the solution did end up outside the mask). Photopolymerisation through the mask was carried out for 3 minutes exposure time. The mask was removed and the surface was rinsed with water. A clear pattern was visible due to the selective wetting at the PAA surface.

Figure 2 illustrates a disc substrate 3 having a hydrophobic surface on which are formed eight 6x5 arrays of hydrophilic spots 1. Figure 3 illustrates a one-dimensional array of hydrophilic spots 1 on a hydrophobic surface 4. As will be explained, with a suitable force applied, a fluid can be caused to pass from spot to spot so that the structure forms a defined channel for fluid flow.

Figure 4 illustrates an arrangement comprising top and bottom plates 5,6 in the form of rotatable discs, having a common axis of rotation. The discs are illustrated far apart, for the purpose of clarity; in practice, the discs will be spaced apart by a distance defined by annular supporting walls 7 which distance will be suitable for the movement of liquid between the plates by capillary action.

The top disc 5 is provided with inlet holes 8 for supplying liquids to the interior. Lining up with these are corresponding areas 9 on the upper surface of the bottom disc 6, which are hydrophilic. Passing in an axial direction between the areas 9 is an elongate area 10, which is also hydrophilic. The remaining parts of the upper surface of disc 6 are hydrophobic. The elongate area 10 effectively forms a channel for liquid between the areas 9. The hydrophilic surface of area 10, bounded on both sides by the hydrophobic upper surface of disc 6 ensures that the liquid pathway is clearly defined by the "walls" which are formed by the interface between the hydrophobic and hydrophilic areas.

If the discs are rotated together about their common axis, it will be seen that centrifugal force will push liquid along the channel formed by area 10 from the innermost area 9 to the outermost area 9.

Figure 5 illustrates how cells might be applied to a hydrophilic area 2. An inlet 23 is provided for introduction of cells and reagent and a hydrophobic channel 24 is provided for respiration of the cells during their growth on the area 2 and for rinsing between tests.

Reference is now made to Figures 6 and 7 which show a microfluidic device in the form of a compact disc (CD) 10 on which are formed hydrophobic and hydrophilic areas to enable liquids to be directed about the surface of the disc to enable the automatic and simultaneous carrying out of multiple chemical/biological tests on multiple samples.

Figure 6 shows a section of the compact disc 10, having a perimeter edge 11, and central hole 12 about which it may be mounted for rotation within a compact disc reader (not shown). On the surface of the compact disc are formed 40 sector-shaped multi-dimensional arrays 16 of hydrophilic spots. As is made clear in the enlarged view A in Figure 7, the spots are arranged in individual straight channels 13 radiating radially from the centre of the disc. Each channel comprises alternate hydrophobic areas or breaks 14 and hydrophilic areas or spots 15. The hydrophobic breaks 14 are typically 75 μm wide in the radial direction. The hydrophilic spots 15 are typically 108 μm wide in the radial direction.

In the illustrated embodiment, there are 20 channels in each array 16 and there are 200 hydrophilic spots 15 in each channel. Thus, each array 16 contains 4000 hydrophilic spots.

The channels in each array 16 begin in a common hydrophilic area 17 and end in a common hydrophobic area 18, constituting a break. Positioned radially outwards from the hydrophobic area 18 is a common waste channel 19.

Liquid reagent for use in carrying out the tests is introduced into an inner annular channel 20 which is common to all of the arrays 16. Extending from the channel 20 are 40 radially extending hydrophobic breaks 21, each extending to the hydrophilic area 17 of a respective array

16. A sample to be tested is introduced into the hydrophilic area 16 at 22. In this way, 40 different samples can be tested simultaneously.

Sample testing is carried out by applying to each of the hydrophilic areas 14 a sample of a known reactant, for example a known oligonucleotide. It will be seen that the device has the potential for testing each sample against 4000 different reactants. A cap may be formed on each hydrophilic spot by evaporation and accurate pre-concentration will occur on vaporisation.

Next the reagent channel 20 is filled and the disc is spun to cause the reagent to jump across the "valve" caused by the hydrophobic break 21 and radially outwardly to the waste channel 19. Progress along the individual channels 13 is by a series of jumps across the effective "valves" caused by the hydrophilic breaks 14. The force required to overcome the breaks is provided by the centrifugal action of the spinning disc.

Once the reagent is issuing into the waste channel 19 the disc is stopped and liquid sample added at 22. Typically the sample volume is 0.1 μl. The disc is now spun at 2 alternating speeds (for hybridisation mixing) whereupon the centrifugal force will move the liquid plug out along channels 13, and capillary action will move the liquid back up. Typically, the sample volume required for each spot 15 is 44 pl.

Reading of the test results is carried out by examining the individual spots 15 using a suitable reader. After the test is completed, the disc may be rinsed by the application of a suitable rinse liquid to the channel 20 and spinning of the disc to move the rinse liquid outwardly along channels 13 by centrifugal force.

Table 1

Surface	Water contact angle (degrees)
Polytetrafluoro-ethylene (Teflon) *	108
Polyethylene *	94
Polypropylene *	95
Polymethyl methacrylate *	80
Platinum *	40
Glass **	"small"
Gold *	65.5

* A C Zettlemoyer (Hydrophobic surfaces, Ed F M Fowkes, Academic Press (New York) 1969, p 1-27

** A W Adamson: Physical chemistry of surfaces 5th ed, Wiley-Interscience 1990, 9-397

CLAIMS

1. A microfluidic device adapted such that the flow of fluids within the device is controlled by different surfaces of the device having different surface characteristics.

2. A microfluidic device according to claim 1 comprising a substrate whose surface is treated to provide areas having different surface characteristics, said areas being arranged to enable control of the flow of fluids passing across the substrate.

3. A microfluidic device according to claim 2 wherein the substrate has a hydrophobic surface interspersed with hydrophilic areas.

4. A microfluidic device according to claim 3 further comprising a second substrate arranged approximately parallel to the first substrate such that fluid entering the device between the substrates will flow along predetermined pathways.

5. A device according to claims 3 or 4 wherein the plurality of hydrophilic areas is an array of hydrophilic spots.

6. A device according to claim 5 wherein the hydrophilic spots are arranged in lines radiating from a central point on the first substrate.

7. A device according to claim 6 wherein the lines of spots are separated by walls connecting the two substrates.

8. A microfluidic device according to claim 2 wherein the substrate has hydrophobic and hydrophilic surface areas which define a pathway for fluid to travel over the surface in which there is at least one hydrophobic/ hydrophilic interface.

9. A microfluidic device according to claim 1, and having predetermined pathways for fluid flow, the surfaces of such pathways being hydrophilic, in which a valve is formed by a section in a pathway having a hydrophobic surface.

10. A microfluidic device according to any one of claims 3 to 9 in which

the surface of at least some of the hydrophilic surfaces is treated to enable the culture of cells.

11. A microfluidic device according to claim 10 which contains gas pathways to enable the access of air to the cell culture.

12. A microfluidic device according to claim 1 wherein the different surface characteristics are defined by different areas of the surface carrying different electrical charges.

13. A microfluidic device according to claim 12 wherein means are provided for changing the charge on the surface to alter the fluid pathway.

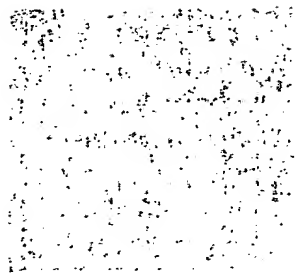
14. A microfluidic device according to claim 1 wherein the different surface characteristics are defined by different areas of the surface being differently magnetised.

15. A microfluidic device according to claim 14 wherein means are provided for changing the magnetisation of the surface to alter the fluid pathway.

16. A microfluidic device according to any of the previous claims which is circular.

17. A microfluidic device according to claim 16 which is adapted for rotation of the device.

18. A microfluidic device according to either one of claims 16 or 17 which has an inlet for fluids towards the centre of the device and an annular outlet for fluids towards the circumference of the device.



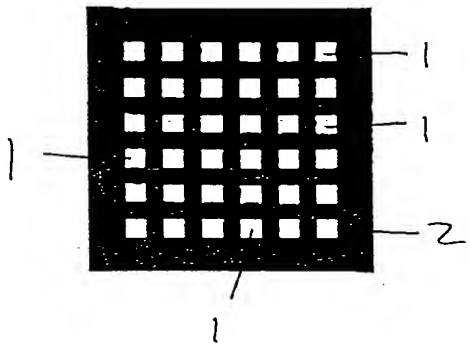


Figure 1

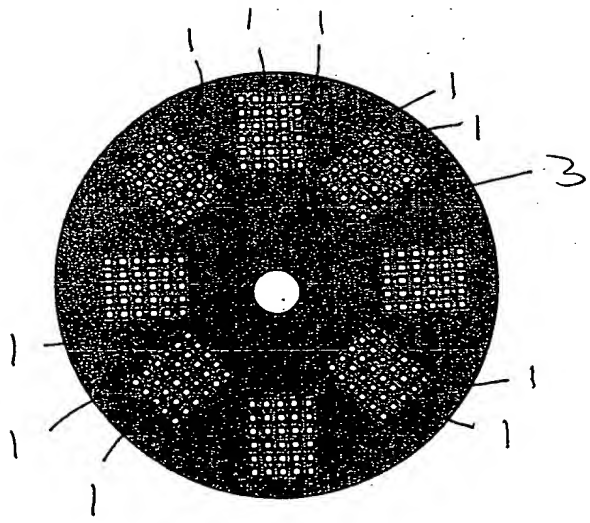


Figure 2

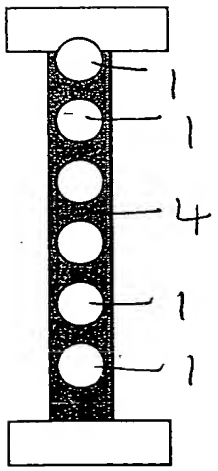
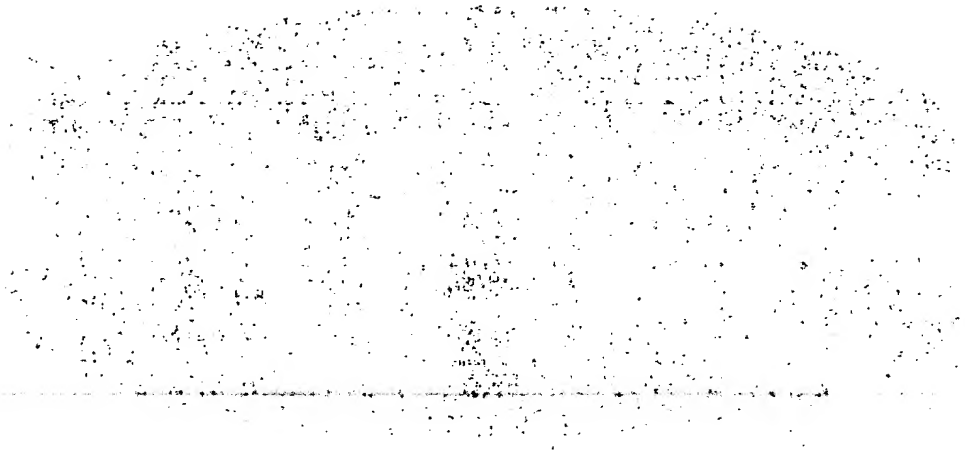


Figure 3



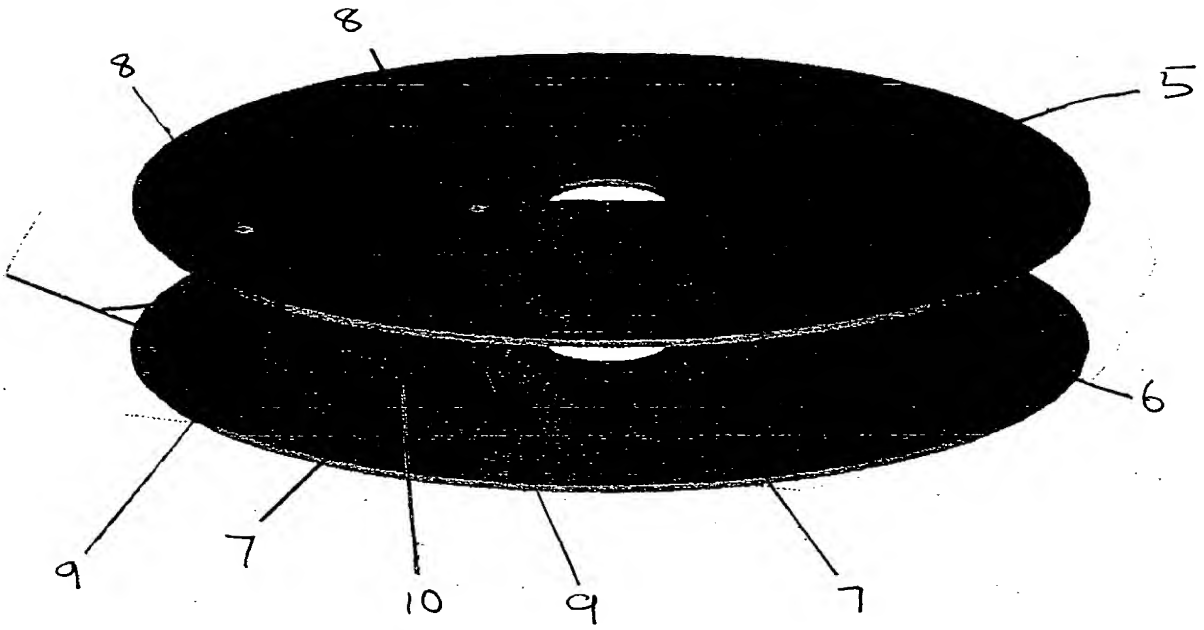


Figure 4

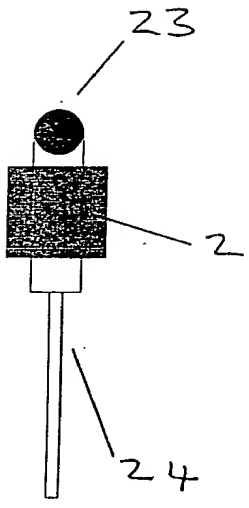


Figure 5

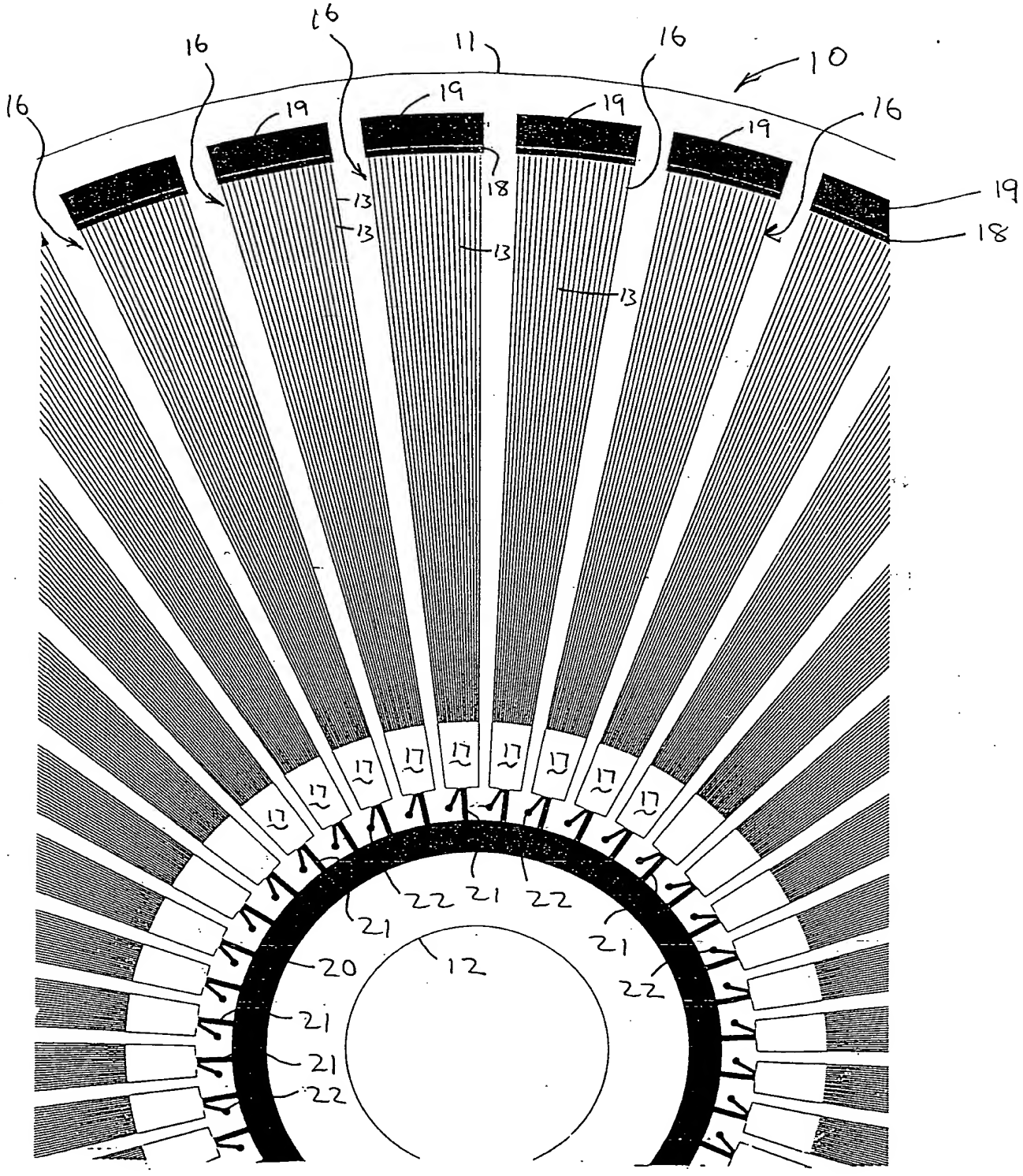

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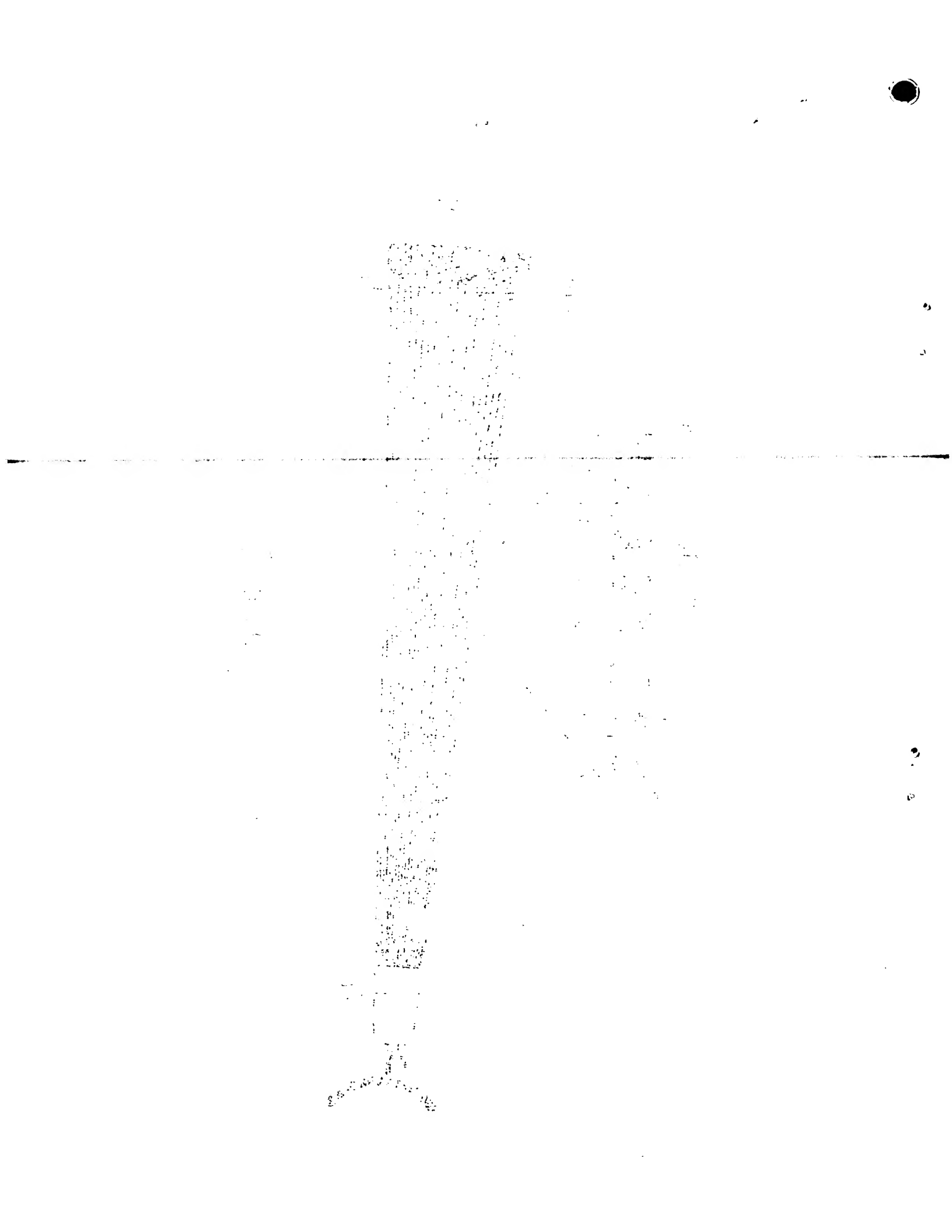
Figure 6



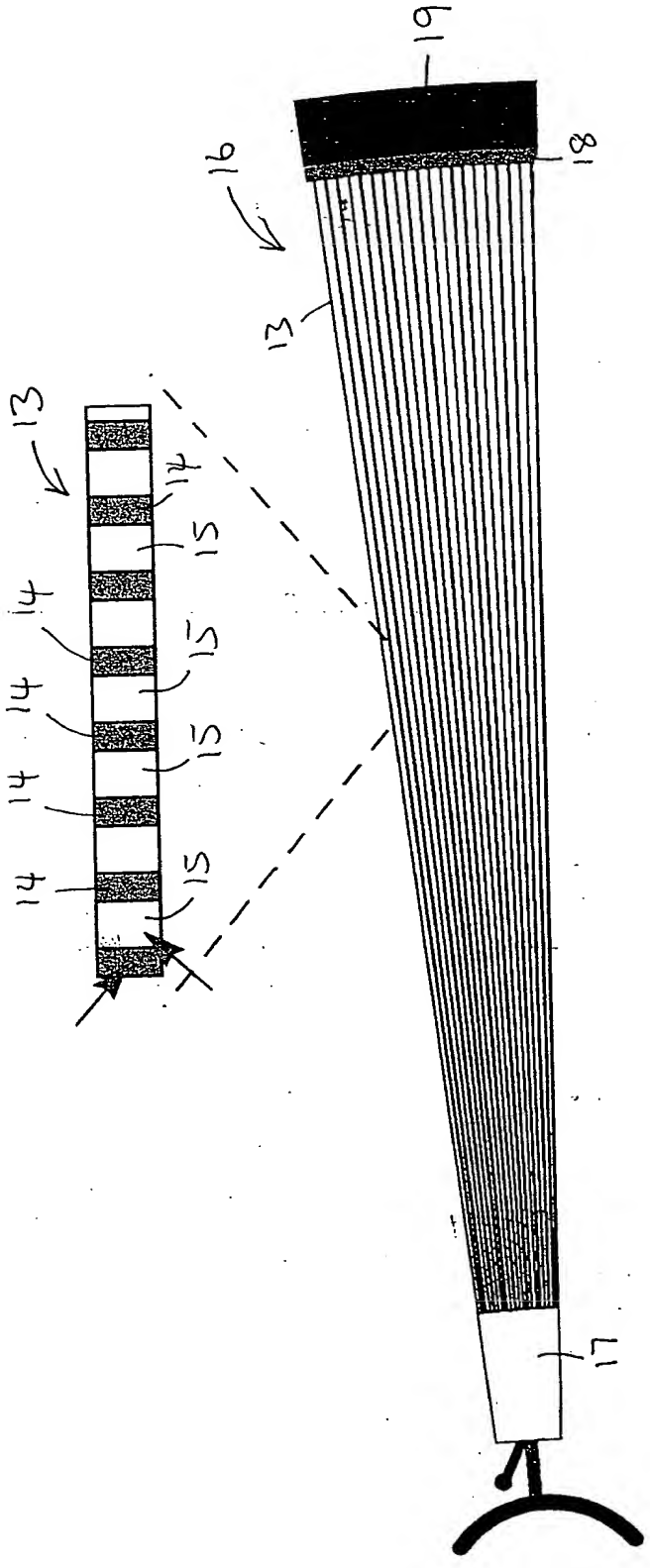


Figure 7